Time course of the antiproteinuric and antihypertensive effect of losartan in diabetic nephropathy

Steen Andersen1, Peter Jacobsen1, Lise Tarnow1, Peter Rossing1, Tina R. Juhl1 and Hans-Henrik Parving1,2

1Steno Diabetes Center, Copenhagen and 2Faculty of Health Science, Aarhus University, Denmark

Abstract

Background. Blockade of the renin–angiotensin system is the primary target in the treatment of diabetic kidney disease. Angiotensin II subtype 1 (AT1) receptor antagonists reduce albuminuria and lower blood pressure, but the initial time course of these effects after initiation of treatment is unknown. We evaluated the time course of the antihypertensive and antialbuminuric effect after initiation of AT1 receptor blockade by losartan in diabetic nephropathy.

Methods. Ten hypertensive type 1 diabetic patients with diabetic nephropathy were included in the study. After a washout period of 4 weeks, patients received losartan 100 mg once daily for 28 days. Every morning, one urine sample was collected for daily determination of albumin-creatinine ratio. Twenty-four hour blood pressure (Takeda TM2420), plasma renin and plasma creatinine were measured at baseline and days 7, 14 and 28.

Results. Baseline levels of urinary albumin-creatinine ratio and 24 h mean arterial blood pressure were 676 (402–1136) mg/g (geometric mean and 95% CI, respectively) and 100 ± 3 mmHg (mean ± SEM). Albumin/creatinine ratio was significantly reduced after 7 days of treatment by 29% (15–41) (95% CI) without significant further reductions during the 28 day study period (P < 0.01 vs baseline). Mean arterial blood pressure was significantly lowered by 7 mmHg after 7 days of treatment and remained unchanged throughout the study (P < 0.01 vs baseline). Plasma renin was significantly increased from baseline after initiation of losartan treatment and stabilized after 7 days (P < 0.01). We found no changes in plasma creatinine during the study.

Conclusions. The initial time course of the reduction in arterial blood pressure and albuminuria are concordant, which suggests that systemic and renal haemodynamic mechanisms are of primary importance in the reduction of albuminuria.

Keywords: albuminuria; diabetic nephropathy; hypertension; losartan; type 1 diabetes

Introduction

Recent studies have demonstrated that angiotensin II subtype 1 (AT1) receptor antagonists represent a valuable new class of drugs in the treatment of diabetic nephropathy [1–3]. Furthermore, the renoprotective effect of AT1 receptor antagonists is suggested to be independent of the beneficial effects on systemic blood pressure. It is well known from studies in diabetic and non-diabetic kidney disease that the magnitude of the reduction in albuminuria after initiation of antihypertensive treatment is a strong predictor of subsequent long-term renoprotection [4,5]. However, the time course of the antihypertensive and antiproteinuric effects after initiation of AT1 receptor antagonist therapy in diabetic kidney disease has never been investigated. Parallel courses of the antihypertensive and antiproteinuric effect would suggest a primarily haemodynamic antiproteinuric response, whereas a dissociation could reflect a contribution from non-haemodynamic mechanisms.

In an attempt to answer these questions, we investigated the time course of the antiproteinuric and antihypertensive effect of losartan 100 mg o.d. in 10 hypertensive type 1 diabetic patients with diabetic nephropathy.

Subjects and methods

Patients

Ten hypertensive patients with diabetic nephropathy were included from the registry of type 1 diabetic patients

Correspondence and offprint requests to: Steen Andersen, Steno Diabetes Center, Niels Steensensvej 2, DK-2820 Gentofte, Denmark. Email: STAN@dadlnet.dk

© 2003 European Renal Association–European Dialysis and Transplant Association
suffering from diabetic nephropathy at Steno Diabetes Center. Diabetic nephropathy was diagnosed clinically in patients with persistent albuminuria (>300 mg/24 h), diabetic retinopathy and absence of other kidney or renal tract disease [6]. Further inclusion criteria were: glomerular filtration rate >60 ml/min/1.73 m², office blood pressure >135/85 mmHg and age 18–70 years. Patients were excluded if they had a history of malignant hypertension, congestive heart failure, myocardial infarction or stroke within the last 3 months.

Design

Before enrolment, all antihypertensive medication was withdrawn for at least 4 weeks. At the end of the washout period, three urine samples from the first morning voiding were collected for determination of baseline urinary albumin/creatinine ratio. Thereafter, the patients received losartan 100 mg o.d. for 28 days. Every morning a sample of the first morning urine was collected for determination of urinary albumin/creatinine ratio. Twenty-four hour ambulatory arterial blood pressure, plasma renin, plasma creatinine and plasma potassium were measured at baseline and days 7, 14 and 28. Dietary intakes of protein or salt were not restricted. Compliance was assessed by pill counts. Patients were to be excluded from the study if compliance was <90%. The study was performed according to the principles of the Declaration of Helsinki and approved by the ethical committee of Copenhagen County. All patients gave their informed consent.

Laboratory procedures

Urinary albumin concentration (turbidimetry; Cobas Mira Plus; Roche) and urinary concentration of creatinine (Cobas Mira Plus; Roche) were measured in all urine collections and the albumin/creatinine ratio calculated.

Plasma creatinine and potassium concentrations were determined from venous blood samples (Cobas Mira Plus; Roche). Haemoglobin A1C (HbA1C) was measured by high-performance liquid chromatography (normal range: 4.1–6.4%) (Variant; Bio-Rad Laboratories). Blood samples for determination of renin concentrations in plasma were drawn after 15 min of supine rest [7,8].

Blood pressure values are based on 24 h ambulatory blood pressure measurements performed with the Takeda TM2420, v. 7 device (A&D, Tokyo, Japan). Blood pressures were measured every 15 min during the day (7.00 a.m. to 11.00 p.m.) and every 30 min during night (11.00 p.m. to 07.00 a.m.). Values were averaged for each hour before calculating the 24 h blood pressure.

Results

All included patients (five male/five female) completed the study. They had a mean age of 41 ± 3 years and diabetes duration of 26 ± 3 years. Mean HbA1C was 9.3 ± 0.4% at baseline. Twenty-four hour arterial blood pressure and urinary albumin/creatinine ratio are given in Table 1. No adverse events that could be related to the study medication were observed.

Urinary albumin/creatinine ratio was significantly reduced after 7 days of treatment by 29% (15–41) (95% CI) (Table 1 and Figures 1 and 2). No significant differences or trends for a second phase of protracted reduction after 7 days were observed. Twenty-four hour systolic and diastolic blood pressures decreased significantly by 12 and 5 mmHg, respectively, after 7 days and remained unchanged thereafter (Table 2 and Figure 1). Separate analysis at baseline of blood pressure during day and night demonstrated values of 149/82 and 136/74 mmHg, respectively. Similar reductions after 7 days were found: systolic blood pressures were lowered by 12 and 13 mmHg and diastolic by 5 and 4 mmHg during day and night, respectively, and remained unchanged throughout the study (P < 0.05). The renin concentration in plasma increased almost 3-fold after 7 days of treatment (P < 0.01) (Table 2). We found no significant changes in plasma creatinine or potassium during the study.

| Table 1. Urinary albumin/creatinine ratio and reduction from baseline in 10 type 1 diabetic patients with diabetic nephropathy after initiation of losartan 100 mg o.d. |
|-----------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
|                            | Baseline            | Day 4               | Day 7               | Day 10              | Day 13              | Day 16              | Day 19              | Day 22              | Day 25              | Day 28              |
| Reduction from baseline (%) | –                   | 25 (17–39)       | 29 (15–41)       | 34 (17–48)       | 33 (18–46)       | 28 (11–47)       | 34 (13–51)       | 28 (9–41)        | 38 (17–54)        | 34 (7–54)        |

Geometric mean (95% CI) of three morning urine samples. All values different from baseline (P < 0.01).

Statistical analysis

Values of urinary albumin/creatinine ratio are given as geometric means of samples from every three consecutive days in Table 1.

Data are expressed as mean ± SEM except for urinary albumin/creatinine ratios, which were logarithmically transformed before statistical analysis owing to their skewed distribution and are given as the geometric means (95% CI). A sample size of 10 patients was based on previous time-course studies with blockade of the renin–angiotensin system (RAS) in patients with diabetic and non-diabetic kidney disease [9,10].

Data are analysed by analysis of variance (ANOVA) according to a general linear model. Subsequently, geometric mean values of urinary albumin/creatinine ratio from three consecutive days are compared with baseline data by the two-sided Student’s t-test. A P-value < 0.05 was considered significant (two-tailed). Data were analysed by SPSS 10.0 (SPSS Inc., Chicago, IL).
Discussion

Our data demonstrate that the maximal antihypertensive and antiproteinuric effects appear within the first 7 days after initiation of losartan treatment. The close match of the time course of the antiproteinuric and antihypertensive effects suggests that the antiproteinuric effect of AT1 receptor antagonism in diabetic nephropathy is primarily related to the systemic and intrarenal haemodynamic changes induced by blockade of RAS. The expected rise in plasma renin concentration after blockade of RAS occurs within 7 days as well. Albuminuria was lowered in nine out of 10 patients. Arterial blood pressure remained unchanged in two patients, including the subject without a reduction in albuminuria (Figure 3). There was no significant correlation between changes in blood pressure and urinary albumin/creatinine ratio ($r = 0.32$, $P = 0.4$). Large variability in the individual response to renoprotective therapy is well known and predicts the long-term beneficial effect of the medication [4,11]. Separate analysis of day and night blood pressure revealed similar reductions during day and night, indicating a 24 h blood pressure control with losartan. This result is consistent with our previous findings in a similar patient population [12].

Our data are consistent with a recent study in nine type 1 diabetic patients with microalbuminuria [10]. A reduction in albuminuria of $\sim 35\%$ was found.

![Fig. 1. Time course of mean arterial blood pressure (mean ± SEM) and urinary albumin/creatinine ratio (geometric mean 95% CI) during treatment with losartan 100 mg o.d.](image1.png)

![Fig. 2. Individual time courses of urinary albumin/creatinine ratio during treatment with losartan 100 mg o.d.](image2.png)

### Table 2. Twenty-four hour blood pressure and laboratory data in 10 type 1 diabetic patients with diabetic nephropathy after initiation of losartan 100 mg o.d.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h systolic blood pressure (mmHg)</td>
<td>$144 \pm 4$</td>
<td>$132 \pm 5^{b}$</td>
<td>$132 \pm 6^{a}$</td>
<td>$130 \pm 4^{a}$</td>
</tr>
<tr>
<td>24 h diastolic blood pressure (mmHg)</td>
<td>$79 \pm 2$</td>
<td>$74 \pm 2^{a}$</td>
<td>$73 \pm 3^{a}$</td>
<td>$73 \pm 2^{a}$</td>
</tr>
<tr>
<td>24 h mean arterial blood pressure (mmHg)</td>
<td>$100 \pm 3$</td>
<td>$93 \pm 3^{a}$</td>
<td>$93 \pm 3^{a}$</td>
<td>$92 \pm 2^{a}$</td>
</tr>
<tr>
<td>P-renin (IU/L)^b</td>
<td>$22 \pm 6$</td>
<td>$63 \pm 21^{a}$</td>
<td>$78 \pm 22^{a}$</td>
<td>$77 \pm 17^{a}$</td>
</tr>
<tr>
<td>P-creatinine (µmol/l)</td>
<td>$104 \pm 9$</td>
<td>$103 \pm 8$</td>
<td>$106 \pm 8$</td>
<td>$107 \pm 8$</td>
</tr>
<tr>
<td>P-potassium (mmol/l)</td>
<td>$4.0 \pm 0.1$</td>
<td>$4.1 \pm 0.1$</td>
<td>$4.1 \pm 0.1$</td>
<td>$4.3 \pm 0.1$</td>
</tr>
</tbody>
</table>

Mean ± SEM. ^a$P < 0.05$ vs baseline. ^b$n = 8.$
after 2 days of treatment with losartan 50 mg, which closely matched the reduction in mean arterial blood pressure over time. The patients were followed for 28 days and albuminuria and arterial blood pressure remained stable throughout the observation period. Conversely, two other studies from the same research group have demonstrated a slower onset of 3–4 weeks of the antiproteinuric effect of losartan and enalapril in patients with non-diabetic kidney disease [9,13]. The reasons for a more prompt antialbuminuric response to RAS blockade in diabetes are unclear. Renal vascular reactivity to angiotensin II may be changed during hyperglycaemia. However, experimental studies have demonstrated conflicting results on that topic [14]. Data from studies in normotensive, uncomplicated type 1 diabetic patients suggest that hyperglycaemia affects renal function by activation of RAS [15], but further studies are needed to assess the intrarenal responses to angiotensin II and AT1 receptor antagonism in diabetes.

Our study does not allow an evaluation of the relationship between the spontaneous variation in ambulatory blood pressure and albuminuria for ethical reasons since patients would have been without antihypertensive treatment for 2 months (washout period). Angiotensin II is a major determinant of glomerular haemodynamics and ultrafiltration [16]. Previous micropuncture studies in streptozotocin diabetic rats demonstrated that prevention of glomerular capillary hypertension by angiotensin-converting enzyme (ACE) inhibition prevented development of proteinuria [17]. Recent human studies in type 2 diabetic patients demonstrated similarly that an increased glomerular pressure may cause albuminuria, which could be reduced by ACE inhibition [18]. This intrarenal haemodynamic mechanism with a close relation between glomerular pressure and albuminuria could be responsible for the concordant time course of the antialbuminuric and antihypertensive effect of AT1 receptor antagonism in the present study. We found no evidence of a protracted antialbuminuric effect, but due to the limited number of patients, a possible variation in the determination of the urinary albumin/creatinine ratio and the observation time of 28 days, we cannot exclude a contribution of slowly onsetting non-haemodynamic mechanisms of AT1 receptor antagonism. Our studies of the glomerular membrane have demonstrated that the size-selective defect for the large shunt-like pores in the glomerular membrane in diabetic nephropathy may be partly restored by treatment with losartan 50 mg o.d. for 2 months [19]. Furthermore, we have previously demonstrated a reduction of urinary IgG excretion of \(~40\%\) during treatment with losartan 100 mg o.d., indicating a reduction in the large pores of the glomerular membrane [1].

Our previous short-term studies with losartan in a similar patient population demonstrated reductions of 24 h mean arterial blood pressure and albuminuria in the same order of magnitude as found in the present study [1,12]. Similar results were found after 2 months with enalapril 10 mg o.d. [1]. Furthermore, our analysis of individual responses to antihypertensive treatment has revealed significant correlations between effects of ACE inhibitors and AT1 receptor antagonists regarding proteinuria and blood pressure [11]. Head-to-head comparison of an ACE inhibitor vs a dihydropyridine calcium antagonist demonstrated a difference in the antiproteinuric effect despite similar blood pressure reduction [20]. It is well known that dihydropyridine calcium antagonists have very limited impact on albuminuria. Obviously, reduction of albuminuria is not influenced only by reduction of systemic blood pressure, but also dependent on intrarenal haemodynamic mechanisms, as discussed above.

A specific antiproteinuric effect of losartan cannot be separated from the antihypertensive effect in the present study. However, it should be noted that angiotensin II is a well-documented growth factor and profibrogenic peptide involved in the pathogenesis of diabetic nephropathy [21]. Therefore, AT1 receptor antagonists may well have long-term non-haemodynamic-related renoprotective effects in addition to the immediate haemodynamic-related effects demonstrated here. Large long-term studies in type 1 and type 2 diabetes have suggested that RAS blockade is associated with a specific antiproteinuric effect above and beyond the systemic blood pressure lowering effect of AT1 receptor antagonists and ACE inhibitors [2,22–24]. Office blood pressure measurements were applied in these studies, but in IRMA-2, 24 h ambulatory blood pressure measurements were performed at our centre in a subset of 43 patients. These data showed identical 24 h ambulatory blood pressure values in the three treatment groups. However, albuminuria was reduced by \(~40\%\) in the high-dose irbesartan group, whereas values remained unchanged in the conventional treated group (S.Andersen, personal communication).

In conclusion, the initial time course of the reduction in blood pressure and albuminuria are concordant, which suggests that systemic and renal haemodynamic mechanisms are of primary importance in the reduction of albuminuria.
Losartan in diabetic nephropathy

Acknowledgements. We acknowledge the assistance of Ms Birgitte V. Hansen, Ms Berit R. Jensen, Ms Ulla Smidt and Ms Inge-Lise Rossing.

References


Received for publication: 4.2.02
Accepted in revised form: 6.9.02